THE RELATION OF THE PITUITARY AND THYROID GLANDS OF BUFO AND RANA TO IODINE AND METAMORPHOSIS.[1]

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In the spring of 1918, Mr. Swingle and Mr. Etzen, working in our laboratory, discovered the fact that iodine administered in loose combination with flour brings about precocious metamorphosis in tadpoles after the same manner as the feeding of thyroid gland preparations previously demonstrated by Gudernatsch, '12. It at once occurred to the writer that iodine administration would afford a means of testing out the rôle of these two glands in metamorphosis. My plan was to administer iodine in this manner to tadpoles deprived of their thyroid glands, to those deprived of the pituitary gland and to those deprived of both. Since the thyroid gland has always been so completely identified with iodine in the animal body, the writer suggested that Mr. Swingle make it a part of his problem to feed iodine to thyroidless tadpoles. This he did with the result that he demonstrated the fact that the administration of iodine by feeding produces metamorphosis just as truly in thyroidless tadpoles as in normal tadpoles. It was shown by the work of Adler, '14, Smith, '16, and Allen, '16, that the removal of the pituitary gland of tadpoles has a marked effect upon the thyroid gland in that the colloid of the latter is of looser texture and smaller amount than normal. Associated with this is the fact that pituitaryless tadpoles do not undergo metamorphosis. A pituitaryless tadpole of *Rana pipiens* has been kept by the writer for two years without undergoing metamorphosis although it has now grown beyond the size normally attained by tadpoles of this species. These facts have led the writer to form the hypothesis that the pituitary gland may play an active rôle in metamorphosis, and that the thyroid gland would play the part of a storage organ. This view was expressed in an earlier paper

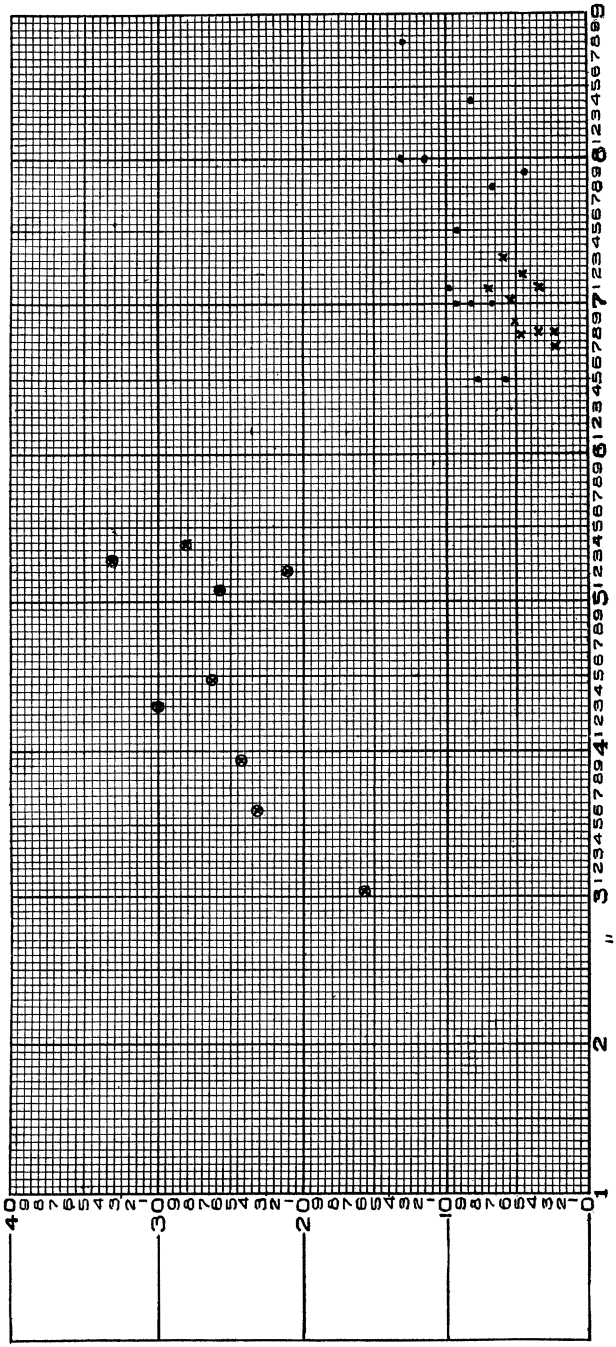

FIG. 1. Showing changes of body length and leg length of pituitaryless *Bufo* tadpoles to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Dots indicate normal control tadpoles. Crosses indicate pituitaryless tadpoles at beginning of iodine feeding. Crosses in circles indicate pituitaryless tadpoles at end of iodine feeding.

(Allen, '17). Swingle's experiments showed that the administration of iodine alone without the mediation of the thyroid gland is able to produce metamorphosis. The peculiar correlation between the thyroid and pituitary glands made it seem highly desirable to carry on experiments in feeding iodine to tadpoles deprived of the pituitary gland and also to those deprived of both the thyroid and the pituitary glands.

The results of this experiment are so definite that they seem well worth recording. A large amount of caution is essential in work of this kind because it has been found that the characteristic color change described by Smith and Allen is not an altogether reliable test of the successful removal of the pituitary gland nor is the failure to metamorphose always to be relied upon as a test of success in the removal of either the pituitary or the thyroid gland. In certain cases, the color change has taken place in spite of the presence of a small remnant of the pituitary gland, and very greatly delayed metamorphosis has been found to take place in tadpoles in which a small fragment of the thyroid gland remained after the operation for removal. Notes along these lines have been published by the writer. For these reasons, it seemed best to make an intensive study of a limited number of specimens rather than to make a necessarily cursory study of a larger number. If the thyroid gland had been removed, the region where it should occur was sectioned and painstakingly studied in order to determine whether the operation had been successful. In the majority of cases where the pituitary gland had been removed, the region involved was likewise sectioned and examined for vestiges of the gland. The work was in this manner checked up to such a degree that the results are offered with full confidence of their accuracy. In these experiments, iodine was mixed with flour in the proportion of 1 to 100. Water was then added to make a creamy paste. This was then dried and the flakes were fed to the tadpoles. In the first series of experiments, *Bufo* tadpoles deprived of their pituitary glands were fed iodine for from ten to twenty-one days. This produced most striking results as shown in Tables I. and II. In this experiment, the tadpoles were kept in the same receptacle and as a consequence it was not possible to follow the changes in

individuals but we must consider mass results. There is a marked decrease in the size of the body. This is out of all proportion to the reduction in body size during normal metamorphosis. There is marked decrease in the length of the tail amounting to complete disappearance in one specimen which was unfortunately not preserved owing to the drying up of the shallow water in which it was necessary to keep it. In ten of the fourteen cases here recorded, both fore limbs had broken through the skin and in three of them the left one alone had appeared.

The difficulties of rearing these larvæ to complete metamorphosis are considerable, but not insurmountable. The small size of the larvæ is no doubt one factor. The sudden marked shrinkage of the body is a decidedly abnormal feature. These results are quite comparable to those attained by Swingle in normal and in thyroidless tadpoles. The removal of the pituitary gland in *Bufo* causes it to assume a light buff color in place of the familiar intense black. This color change was in no wise modified by the subsequent iodine feeding. Fig. 1 shows in graphic fashion the nature of these changes as regards the typical feature of hind leg length. The abscissæ represent the length of body while

TABLE I.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.			No.	Iodine Feeding Begun May 31, 1918, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
14.8	6.9	.50	1.	9.3	3.93	5.37	2.44	L.	21
14.6	7.2	.46	2.	5.9	3.60	2.34	2.34	R. L.	21
12.6	6.8	.22	3.	8.2	4.29	3.91	3.00	L.	28
13.0	6.7	.32	4.	9.2	4.49	4.71	2.67	R. L.	18
14.2	7.1	.76	5.	9.6	5.08	4.52	2.57	R. L.	18
16.1	7.3	.59	6.	7.5	3.03	4.47	1.52	R. L.	17
14.5	6.8	.46	7.	9.9	5.38	4.52	2.80	R. L.	18
14.4	7.1	.34	8.	11.3	5.28	6.02	3.30	R. L.	18
13.9	6.8	.31	9.	11.6	5.20	6.40	2.10	....	10
15.1	7.0	.54							
14.3	6.97	.44		9.18	4.67	4.69	2.59		

the ordinates represent the length of the hind legs. The location of any point serves to indicate the length of body by its horizontal location and the length of the hind legs by its height above

the base line. Dots are used to designate control, normal tadpoles; crosses designate the pituitaryless tadpoles just before the administration of iodine while crosses within circles indicate the pituitaryless tadpoles at the end of the period of iodine feeding. It appears that the pituitaryless tadpoles at the beginning of the experiment are at this stage already commencing to undergo a retardation in hind leg growth as compared with the normal controls. In the iodine fed, pituitaryless tadpoles the reduction in body length together with an actual and relative increase in leg length are clearly evident.

The next series of experiments involved feeding iodine in the same manner to tadpoles from which both the pituitary and thyroid glands had been removed. In this case, tadpoles were kept separated in different dishes so that it was possible to follow the growth of each individual tadpole. Unfortunately, all died within ten days. This may have been partly due to the excessively warm weather at the time. Because of the short period

TABLE II.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.				No.	Iodine Feeding Begun June 18, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
20.6	9.8	10.8	.93	10.	10.7	6.4	4.30	3.17	R. L.	18
18.8	9.7	9.1	.88	11.	9.1	4.55	4.55	2.05	L.	18
18.5	8.8	9.7	.51	12.	10.3	4.82	5.48	2.41	R. L.	23
15.6	8.4	7.2	.53	13.	8.2	5.15	3.05	2.57	R. L.	R. L.
14.8	7.3	7.5	.38		9.6	5.23	4.34	2.55		
18.8	8.7	10.1	1.37							
17.8	8.8	9.1	.77							
					Iodine Feeding Begun June 3.					
				14.	9.3	6.34	2.96	3.60	R. L.	15

of the experiment, the results are not so striking as one might desire but they point in the same direction as the other experiments and are quite significant. Table III. gives the results and needs no further explanation. Table IV. gives the dimensions of *Bufo* tadpoles deprived of both thyroid and pituitary glands but maintained under normal food conditions. A comparison of the tables shows that these do not have hind legs of length at all comparable with those that were fed iodine, even though the

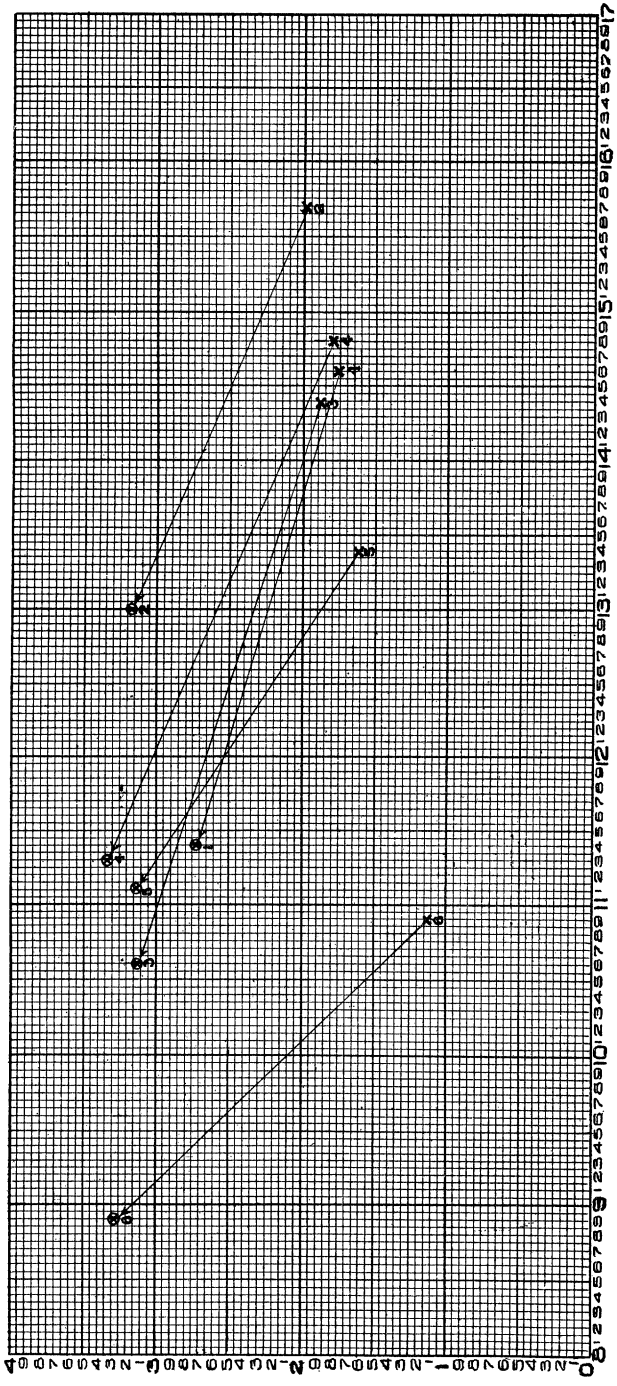

FIG. 2. Showing modifications of body length and leg length of pituitary-thyroidless tadpoles of *Bufo* to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Arrows connect the points indicating any given individual at the beginning and the end of the experiment. The numbers of the points correspond to the numbers of Table III.

TABLE III.

Bufo PITUITARY-THYROIDLESS TADPOLES.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Sucess of Operation.	Length of Feeding.
1.	33.1	14.6	18.5	1.75	No pituitary. No thyroid.	7 days.
2.	33.4	15.7	17.7	1.95	No pituitary. No htyroid.	10 days.
3.	32.5	14.4	18.1	1.91	No pituitary. No thyroid.	10 days.
4.	32.6	14.7	17.9	1.78	No pituitary. No thyroid.	10 days.
5.	29.1	13.4	15.7	1.62	Minute vestige of pituitary. No thyroid.	10 days.
6.	25.8	10.9	14.9	1.12	No pituitary. No thyroid.	10 days.
	31.2	13.9	15.5	1.69		

IODINE FEEDING BEGUN JULY 8.

Condition at End of Experiment.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.
1.	26.2	11.4	14.8	2.71
2.	25.1	13.0	12.1	3.17
3.	26.1	10.6	15.5	3.13
4.	27.2	11.3	15.9	3.33
5.	27.9	11.1	16.8	3.14
6.	18.5	8.9	9.6	3.27
	25.2	11.0	14.3	3.12

tadpoles represented in Table IV. were killed about two and one half months later and were then not only of larger size but older as well. Data are accumulating to show that the hind limbs continue to grow proportionately longer in older specimens than in younger ones of equal size. This seems to be generally true of thyroidless, of pituitaryless and of thyroid-pituitaryless tadpoles. More data upon this point must be gathered before a final conclusion can be reached because there are decided individual differences in the rate of limb growth in normal and operated tadpoles under apparently identical food conditions. In any case, a comparison of Tables III. and IV. will serve to answer any possible objections upon this score.

Fig. 2 shows the modifications of body length and leg length

indicated in Table III. The numbers of the points apply to the same individuals shown in the table. The arrow lines connect up points showing the dimensions of each specimen at the beginning and at the end of the feeding.

TABLE IV.

Bufo PITUITARY-THYROIDLESS TADPOLES.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	20.4	9.8	1.75 K.	Sept. 24.
2.	23.0	10.9	2.14 K.	Sept. 24.
3.	24.1	11.3	2.54 K.	Sept. 26.
4.	26.1	11.4	2.41 K.	Sept. 24.
5.	25.4	13.2	1.91 K.	Oct. 31.
	23.8	11.2	2.15	

Table V. shows the results of feeding iodine to *Rana pipiens* tadpoles from which both the thyroid and pituitary glands had been removed. The experiment was begun with five specimens, two of which were not preserved. These five tadpoles at the beginning of iodine feeding had body lengths ranging from 10.3 mm. to 15.2 mm. and the hind legs ranged in length from .28 mm. in the smallest to .80 mm. in the largest. It is probable that no. 1 in Table V. represents the largest and no. 3, the smallest but it is not possible to be certain upon this point because the tadpoles were all kept together in the same aquarium. In any case, the growth of the hind legs is remarkable and it will be

TABLE V.

Rana pipiens WITH PITUITARY AND THYROID GLANDS REMOVED.
Fed Iodine from June 30 to July 25.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Fore Limbs.	Remarks.
1.	23.5	10.4	6.04	None	No thyroid. No pituitary.
2.	19.8	9.1	4.16	Left free	No thyroid. No pituitary.
3.	17.1	7.2	2.77	Left free	No thyroid. No pituitary.

noted that the left fore limb has appeared in nos. 2 and 3. The heads of these three tadpoles were sectioned and the series carefully studied in order to detect any remnants ever so small of thyroid or pituitary glands. Not the slightest vestiges of these

were found. In Table VI. the hind leg lengths of pituitary-thyroidless tadpoles normally fed and killed more than a month

TABLE VI.

Rana pipiens WITH PITUITARY AND THYROID GLANDS REMOVED.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	23.7	10.2	.43 K.	Aug. 22.
2.	28.3	11.2	.53 K.	Aug. 22.
3.	49.0	19.5	.76 K.	Oct. 7.
4.	59.8	23.3	2.28 K.	Aug. 21.

later than the above are found to be in sharp contrast to these. None of these pituitary-thyroidless *Rana pipiens* tadpoles were brought to metamorphosis by iodine feeding but there is little doubt but that it will be possible to do so; at any rate there is an extremely strong tendency in that direction.

TABLE VII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES—IODINE FED. NUMBERS OF SPECIMENS CORRESPOND TO THOSE OF TABLES I. AND II.

Dimensions of Thyroid Gland.

No.	Total Length, Mm.	Body Length, Mm.	Side Length, Mm.	Width, Mm.	Thickness, Mm.	Volume, Cmm.	Aver. Vol., Cmm.	Aver. Diam. Colloid Mass, Mm.
1.	9.3	3.93	R. .100	.064	.031	.000198	.000209	.014
			L. .100	.071	.031	.000220		.014
3.	8.2	4.29	R. .137	.080	.037	.000405	.000409	.012
			L. .157	.080	.033	.000414		.014
7.	9.9	5.38	R. .149	.090	.036	.000482	.000364	
			L. 118.	.070	.030	.000247		
8.	11.3	5.28	R. .086	.070	.046	.000276	.000275	.024
			L. .078	.080	.044	.000274		.026
9.	11.6	5.20	R. .146	.090	.029	.000381	.000396	.016
			L. .089	.140	.033	.000411		.014
12.	10.3	4.82	R. .113	.070	.027	.000213	.000444	.014
			L. .157	.100	.043	.000675		.020
13.	8.2	5.15	R. .193	.100	.043	.000710	.000547	
			L. .150	.080	.032	.000384		
14.	9.3	6.34	R. .190	.121	.040	.000899	.001324	.016
			L. .220	.150	.053	.001749		.016
	9.8	5.04					.000471	.016

The iodine-fed pituitaryless *Bufo* tadpoles shown in tables I. and II. were sectioned and their thyroid glands studied, with the object of seeing whether the iodine feeding had brought about

any notable increase of colloid in the thyroid gland. Two were preserved intact for demonstration purposes. Only eight out of the twelve tadpoles sectioned were found to be in a satisfactory state of preservation for histological study. This was due to the fact that the tadpoles were usually preserved after they had died. Although the aquaria were frequently examined, the summer heat often caused considerable disintegration of the thyroid gland before the specimens could be preserved. An examination of Table VII. shows the length, width and thickness

TABLE VIII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES NORMAL FEEDING AS CONTROLS:

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Side Length, Mm.	Breadth, Mm.	Thickness, Mm.	Volume, Cmm.	Average Volume, Cmm.	Diameter of Colloid Masses, Mm.
1.	16.7	7.4	.92	R. .110	.117	.047	.000604	.000469	.013
				L. .130	.081	.069	.000335		
2.	13.7	6.6	.36	R. .060	.150	.076	.000684	.000523	.011
				L. .070	.089	.060	.000373		.010
3.	17.8	8.7	1.09	R. .130	.111	.057	.000822	.000791	.011
				L. .150	.118	.043	.000761		.016
4.	13.6	6.4	.40	R. .090	.110	.078	.000722	.000679	.011
				L. .090	.107	.061	.000587		.011
5.	14.0	6.7	.40	R. .070	.080	.036	.000201	.000230	.013
				L. .090	.072	.040	.000259		.013
6.	14.0	7.0	.56	R. .180	.094	.041	.000693	.000629	.014
				L. .160	.104	.034	.000565		.014
Aver	14.6	7.1						.000554	.013
7.	22.5	10.5	3.23	R. .330	.193	.057	.003330	.003382	.024
				L. .339	.153	.068	.003435		.024
8.	26.8	13.4	4.16	R. .460	.236	.057	.006187	.006770	.028
				L. .490	.224	.167	.007353		

of each thyroid gland. The specimens are designated the same as in Table I. From the dimensions of each gland was calculated the volume of a parallelopiped that would contain it. This of course would constitute but a rough relative approximation to the volume. Table VIII. shows similar measurements and calculations made upon the thyroid glands of normally fed pituitaryless *Bufo* tadpoles of size corresponding to these as measured at the beginning of iodine feeding. A comparison of Tables VII. and VIII. will show that the thyroid glands taken as

a whole have shrunken appreciably together with the shrinkage of the body as a whole; but not proportionately as much. In the iodine-fed tadpoles the colloid masses show a slightly greater average diameter than do those of the normally fed specimens. These facts do not seem especially significant. The slight increase of the colloid masses and the actual decrease in the dimensions of the gland roughly counterbalance one another. It is to be admitted that the number of specimens is small but it is large enough to demonstrate that no striking changes in the size of the thyroid glands and of their colloid content in pituitaryless tadpoles result from iodine feeding, at least, not within the stages and time limits of the experiment. It will be interesting in future experiments to test whether feeding iodine at early stages prior to the normal time of colloid formation would cause it to appear precociously.

In a paper upon the "Development of the Thyroid Glands of *Bufo* and their Normal Relation to Metamorphosis," Allen (in press), a study was made of the relation of the growth of the thyroid gland to the growth and metamorphosis of *Bufo* tadpoles. The stages used are a trifle more advanced than those used in this experiment. It appears, however, that the thyroid glands are of about the same size in their early stages in pituitaryless tadpoles as in normal tadpoles and that the colloid begins to form at the same time in both.

Summary and Conclusions.

At the beginning of this paper, attention was called to the fact that the growth of the thyroid glands and the accumulation of colloid in them are retarded by the removal of the pituitary gland. Rogers and Larson have both shown that the removal of the thyroid gland causes an hypertrophy of the pituitary gland (Rogers, '18, anterior lobe; Larson, '19, anterior and intermediate lobes). It would be premature to offer an interpretation of these facts but they seem to justify the hypothesis that these glands are closely interrelated. The writer has for several years favored the hypothesis that the thyroid gland may, largely at least, play the rôle of a storage organ for the iodine of the body, at the same time controlling its distribution. It even seems

possible that the pituitary gland might play the chief rôle in the utilization of iodine by the body. This view is in the main based upon the fact that tadpoles of *Rana* and *Bufo*, deprived of the pituitary gland, fail to metamorphose in spite of the fact that the thyroid glands remain intact and even though the body of the tadpole reaches gigantic size. The removal of the pituitary gland renders the thyroid gland as powerless to bring about metamorphosis as though the thyroid gland were itself removed.

It was shown by Swingle that metamorphosis could be produced in normal tadpoles by feeding them iodine. He likewise showed that iodine feeding would produce metamorphosis even in tadpoles deprived of the thyroid glands. Simultaneously with the above work, the writer was carrying on the experiments that form the subject of the present paper. They show conclusively that metamorphosis can be produced by iodine feeding in tadpoles deprived of the pituitary glands. More striking still is the demonstration of the fact that feeding iodine to tadpoles deprived of both thyroid and pituitary glands can carry them far in the process of metamorphosis, the attainment of which was prevented only by the death of the tadpoles.

In the light of all these facts, we are justified in drawing the following general conclusions:

With normal feeding, involving the intake of very minute quantities of iodine, the presence of both the thyroid and pituitary glands is essential to enable the animal to undergo metamorphosis. Unpublished data show quite conclusively that *Rana* and *Bufo* larvæ proceed to the same stages of limb growth in the absence of the thyroid or of the pituitary gland or of both together. The administration of large quantities of iodine will cause the tadpoles to undergo metamorphosis in the total absence of the thyroid gland or of the pituitary gland or in the simultaneous absence of both thyroid and pituitary glands. It may be found by later experimentation that iodine is not the only factor that is capable of bringing animals to maturity. Investigation along these lines is still in its infancy. Before this paper appears in print, the writer expects to have under way more extensive quantitative experiments that will determine the reactions of normal, pituitaryless, thyroidless, and pituitary-

thyroidless tadpoles to these and to other factors that influence development.

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